

ACCURACY AND REPEATABILITY OF PROTEIN CONTENT MEASUREMENTS FOR WHEAT DURING STORAGE

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ABSTRACT. Producers with wheat stored on-farm for a few months are concerned about unexpected decreases in protein content measurements obtained from commercial laboratories. These differences can adversely affect the price when the wheat is sold. This study evaluated the contribution of measurement errors in giving a false indication of protein change during storage. Eleven bins of wheat were sampled at three in-bin positions during one storage season and five of these bins were refilled and sampled during the second season to evaluate differences in protein measurements. Samples were analyzed for protein content using four measurement instruments. Additional wheat was stored in the laboratory and evaluated over two years with two instruments. Data showed that the variation between protein measuring instruments was significant with an expected variation of $\pm 0.74\%$ protein content (95% confidence interval) during the field tests. The variation over time for measurements with the FGIS instrument was $\pm 0.3\%$ protein for an eight-month period, when measuring successive samples taken from the same positions. Measurements from the other three instruments varied by $\pm 0.8\%$ protein or more during the same time. Variation with in-bin position was not significantly different ($\alpha = 0.05$) than the variation between instruments. The greater consistency for the FGIS instrument was likely due to the rigorous standardization and maintenance procedures employed by FGIS for their near-IR protein instruments. These results suggest that a similar rigorous system is needed to obtain the same consistency for other instruments used in the wheat marketing system.

Keywords. Protein content, Wheat, Grain storage, Grain quality, Near-infrared, NIR, Standardization, Measurement error, Repeatability.

There have been anecdotal reports of wheat stored on-farm for a few months showing large decreases in protein content (up to 2%) based on measurements from commercial laboratories (Suchan, 1997). Wheat prices, such as for hard wheat, may be discounted (or lose a premium) if the grain has a low protein content. The reverse situation, discounting for high protein, may occur with some soft wheat. Thus, reports of wheat protein content measurements showing a decrease during storage are a serious concern for producers that store their wheat.

A review of the existing literature about protein changes during storage shows that protein quality loss has been measured during storage, especially at elevated storage temperatures (Pomeranz, 1992). Pixton and Hill (1967) reported that wheat stored under typical commercial storage

conditions for eight years showed no change in crude protein content. Daftary et al. (1970) studied badly mold-damaged wheat and found that the protein content (percent) was slightly higher although the proteins were damaged. The percentage increase was entirely explained by carbohydrates lost through respiration. Jones and Gersdorff (1941) reported that wheat stored in jars for two years showed decreased protein solubility, decreased true protein nitrogen (-10%), and no change in total nitrogen. They attributed the decrease in true protein nitrogen to enzymatic activity. Protein quality changes in corn have been observed in the short term during drying at elevated temperatures, but not at room temperature in that short time (Peplinski et al., 1994). Lukow et al. (1995) reported no significant protein content change in two cultivars of hard red spring wheat during 15 months of storage.

Williams (1975) found a standard error of estimate of 0.22% protein with early near-infrared (near-IR) reflectance instruments using ground wheat samples, while Williams (1979) predicted the protein content within 0.31% with this type of ground sample instrument. Williams et al. (1982) found significant errors in protein measurements caused by the temperature increase during the grinding process during this type of near-IR analysis and presented a method to alleviate this problem. Delwiche et al. (1998) more recently evaluated four whole grain near-IR reflectance and transmittance instruments and found standard errors ranging from 0.18 to 0.24% protein content.

It is generally agreed that total protein content as indicated by nitrogen theoretically cannot change during storage if there is no microbial activity. Without mold problems, there is no known mechanism for protein loss and sampling or

Article was submitted for review in January 2002; approved for publication by the Food & Process Engineering Institute Division of ASAE in December 2002. Presented at the 1998 ASAE International Meeting as Paper No. 986029.

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measuring error is a more likely problem. However, the suggested enzymatic activity by Jones and Gersdorff (1941) indicates that protein quality does change. With these concerns, care must be taken with sampling and measurement techniques to ensure accurate data on true protein changes. This two-year survey of protein measurements in grain stored under typical conditions will clarify the reports of protein losses in storage, and help identify the role of measurement instruments in determining or indicating losses.

OBJECTIVES

The purpose of this research was to evaluate the contribution of measurement errors during routine measurements in the field in giving a false indication of protein change during storage. Specific objectives were to: 1) determine the range of protein readings from measurements in stored wheat over a typical storage season for four common protein instruments, 2) evaluate the differences in protein readings between the four instruments, and 3) compare the differences in instrument readings with the sample-to-sample variation in protein content.

PROCEDURES

Eleven storage bins, belonging to project cooperators, were sampled during the 1996–1997 storage season. Two of the bins were in the Moscow, Idaho area and the remaining nine were in the Aberdeen, Idaho area, with a mixture of aerated and non-aerated bins. Five of these bins near Aberdeen were refilled and sampled again during the 1997–1998 storage season. Most of the bins were sampled three times in 1996, and then the grain was sold after the measurements at five months of storage. One bin was sampled a fourth time after eight months. In 1997, all bins were sampled twice before the grain was sold.

Samples were analyzed for protein content using four measurement instruments. For these bin samples, both a ground sample near-IR instrument, Instalab 600 NIR Product Analyzer (Dickey-John Corporation, Auburn, Ill. —referred to herein as Lab-NIR), and a combustion nitrogen analyzer (Leco Corporation, St. Joseph, Mich. —referred to herein as LECO) were used at the University of Idaho Wheat Quality Laboratory in Aberdeen. For these instruments, 40 g of kernels were ground and mixed, with 2 g used for the Lab-NIR instrument and used 0.25 g for the combustion nitrogen analysis. The sample was dropped twice in the Lab-NIR and the average of the two readings recorded. The bias of the Lab-NIR was regularly adjusted based on the LECO measurements. In addition, whole grain near-IR instruments was used for measurement at local elevators (500-g sample) and by the Federal Grain Inspection Service (FGIS) (minimum 500-g sample). The elevator used an Infratec 1221 (Foss North America, Eden Prairie, Minn. —referred to herein as Elevator-NIRT) and FGIS used a Tecator-Infratec 1226 (Foss North America —referred to herein as FGIS-NIRT). Samples were dropped once for both of these instruments. The laboratory and elevator instrument operators indicated that these instruments were maintained and calibrated according to manufacturer instructions and, in the case of the elevator instrument, calibration was according

to state of Idaho standards. The FGIS procedures are detailed in the NIRT Handbook (USDA–GIPSA–FGIS, 1999).

While the standard error of performance (SEP) is not known for these specific instruments and calibrations, Delwiche et al. (1998) reported data that provide expected SEP values for both the Infratec 1221 and the Tecator-Infratec 1226. In a large collaborative study they found an average SEP of 0.178 based on results from 10 instruments, which were Tecator Infratec models 1221, 1225, or 1226.

Test bins were sampled at three to five positions as shown in figure 1. All the bins were initially sampled during the first two weeks after harvest. Grain samples were taken at three depths with a deep-bin probe in the bin center in all cases. For the two bins in Moscow, samples were also taken at the north and south radii of the bin, 0.3 m from the bin wall during the first year. At the center of all bins, samples were taken at depths of 0.5, 1.5, and 2.5 m. The samples near the wall were taken at a depth of 1.5 m. Grain moisture contents were also determined by FGIS and with the near-IR instruments. In 1996 moisture contents ranged from 7.6 to 10.9% wet basis. In 1997 they ranged from 9.2 to 12.8%. All protein measurements used in this study were corrected to a constant 12% moisture basis. Table 1 shows the test bin characteristics.

Two additional specimens were taken from each of the bins in the study initially and stored in sealed glass jars at two different temperatures (5°C and 22°C). The lower temperature simulated an aerated bin, while the higher temperature corresponded to a non-aerated bin. The samples were checked for protein content using the Lab-NIR (which also checked moisture content) and the combustion nitrogen analyzer at the Wheat Quality Laboratory eight times over a period of 22 months.

Data were analyzed by averaging and plotting the protein content readings over time, over measurement instrument, and over bin position. The averages and standard deviations (S.D.) were evaluated to compare the different instruments. In these cases, the percent coefficient of variation was also calculated as: $\% \text{ C.V.} = (\text{standard deviation})/(\text{mean})$. These coefficients of variation were used as a measure of instrument variability and they were analyzed by analysis of variance using the SAS procedures ANOVA and GLM as appropriate for balanced and unbalanced data, respectively. Means were compared using Duncan's new multiple range test. All analyses of variance and comparisons are reported at the 5% level of significance ($\alpha = 0.05$)

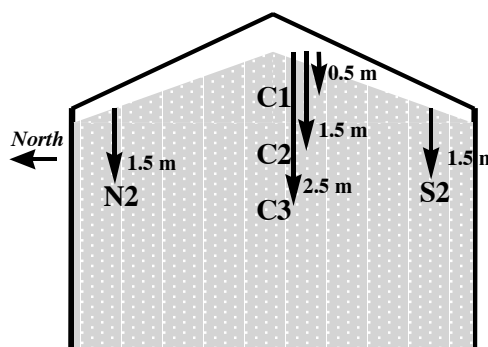


Figure 1. Bin sampling positions.

Table 1. Bins sampled for protein measurement.

Bin	Location	Bin Capacity (bu)	Wheat Class	Aeration	Sample Points	
					1996	1997
A	Aberdeen	5,000	HRW	no	3	3
B	Aberdeen	5,000	HRW	no	3	3
C	Aberdeen	5,000	SWH	yes	3	3
D	Aberdeen	5,000	HRW	no	3	3
E	Aberdeen	10,000	HRS	no	3	—
F	Aberdeen	5,000	HRW	no	3	3
G	Aberdeen	5,000	HRS	no	3	—
H	Aberdeen	10,000	HRS	no	3	—
I	Moscow	10,000	SWH	yes	5	—
J	Moscow	10,000	SWH	no	5	—
K	Aberdeen	10,000	SWH	yes	3	—

RESULTS AND DISCUSSION

IN-BIN MEASUREMENTS

Protein results for the 8-month bin in 1996 are shown in figure 2. Results from all four instruments are shown in the graphs for the three positions in the center of the bin (positions C1, C2, and C3). The FGIS (whole grain) near-IR measurements showed less variation over time than the other three instruments, the lab (ground sample) near-IR machine, the combustion nitrogen analyzer, and the (whole grain) near-IR machine at a local elevator. This difference was typical of the results seen for all bins as indicated by the data in tables 2 and 3, which show that the FGIS-NIRT instrument had significantly less variation over time than the other two near-IR instruments during both years of the study.

Tables 2 and 3 summarize the average standard deviations for each instrument when the protein content was averaged over time. Thus, the statistics in table 2 and 3 indicate variation in each instrument plus any sample-to-sample variation when sampling from the same position. The standard deviations for the FGIS-NIRT measurements were lower than for any other instrument both years. For one case, the combustion nitrogen analyzer in 1997, the difference was not significant but the FGIS-NIRT values were always significantly lower than the other near-IR instruments. In 1996, readings from the FGIS-NIRT instrument were only

obtained at two time intervals for 7 of the 11 bins (three time intervals for the other four), while the other three instruments had readings from at least three time intervals. The standard deviation statistic is an unbiased estimator that is not biased by a difference in sample size. However, because the FGIS instrument measurements occurred over a shorter period in 1996, there was less possibility of instrument drift affecting the measurements from that instrument. The 1997 data, which had only two time intervals for all instruments, also showed less variation for the FGIS instrument than the others; the % C.V. mean for the FGIS-NIRT was again significantly different than the other two near-IR instruments during 1997.

When comparing the protein measurements from the different instruments, it was normal for at least two of the instruments to differ by about 0.5 to 1.0% protein content at the same time and in-bin position. Differences of almost 2% protein content were recorded. These differences resulted in standard deviations up to about 1% protein content, which corresponds to coefficients of variation of up to 12%, and averaging 4.9% for all in-bin positions in 1996 as seen in table 2. Note that tables 2 and 3 indicate variation over time only. Assuming little change in total protein content, this variation was largely for the individual instruments due to some variation in procedures (e.g., lack of standardization) or equipment (e.g., instrument drift), but also including sample variation. The repeatability and stability in these field tests were not on par with that reported in the large study of laboratories by Delwiche et al. (1998) except for the FGIS-NIRT instrument.

There were three distinct sources of variation that contributed to these standard deviations for the individual instruments: 1) sampling variation from differences in adjacent grain at individual sample positions, 2) instrument and procedural errors, and 3) changes in protein content of the grain. Based on the literature review, it is likely that the third source was negligible because the samples were not moldy. That the standard deviations in the laboratory samples (table 7) were the same or higher than these from in-bin tests indicates that the first source, sampling variation, was also small. In any case, the higher standard deviations for the other three instruments indicated a greater instrument error than with the FGIS instrument, because the other sources of error must have been the same.

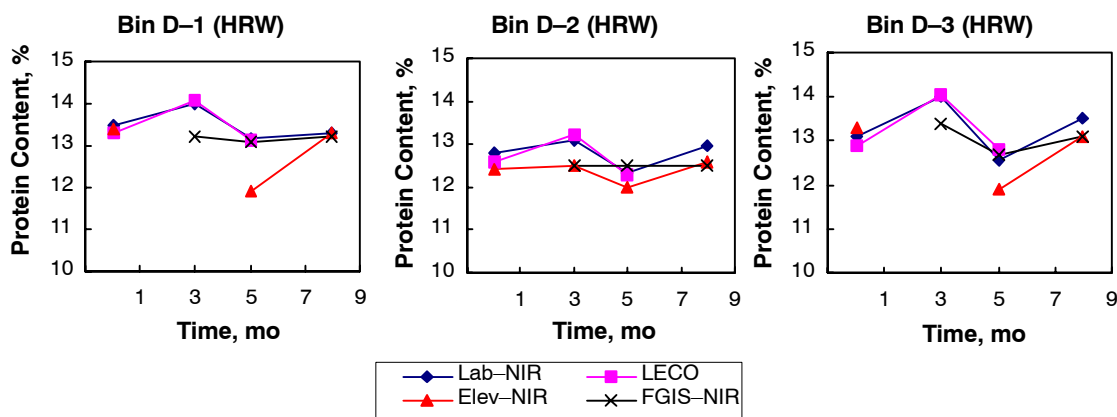


Figure 2. Protein measurements from wheat stored eight months, 1996 (three positions in one bin).

Table 2. In-bin percent protein content statistics for all instruments averaged over time, 1996.

Instruments	N	Std. Dev.	95%	% C.V.[a]
Lab-NIR, 1996	30	0.634	± 1.24	5.93 ^a
LECO, 1996	24	0.403	± 0.79	3.65 ^a
Elevator-NIRT, 1996	9	0.559	± 1.10	5.45 ^a
FGIS-NIRT, 1996	22	0.161	± 0.31	1.39 ^b
Combined Instruments, 1996	85	0.532		

[a] Mean values of % C.V. with a different letter beside them were significantly different by Duncan's new multiple range test ($\alpha = 0.05$).

Table 3. In-bin percent protein content statistics for all instruments averaged over time, 1997.

Instruments	N	Std. Dev.	95%	% C.V.[a]
Lab-NIR, 1997	15	0.251	± 0.49	2.32 ^a
LECO, 1997	15	0.107	± 0.21	0.95 ^b
Elevator-NIRT, 1997	15	0.222	± 0.43	2.08 ^a
FGIS-NIRT, 1997	15	0.080	± 0.16	0.76 ^b
Combined Instruments, 1997	60	0.165		

[a] Mean values of % C.V. with a different letter beside them were significantly different by Duncan's new multiple range test ($\alpha = 0.05$).

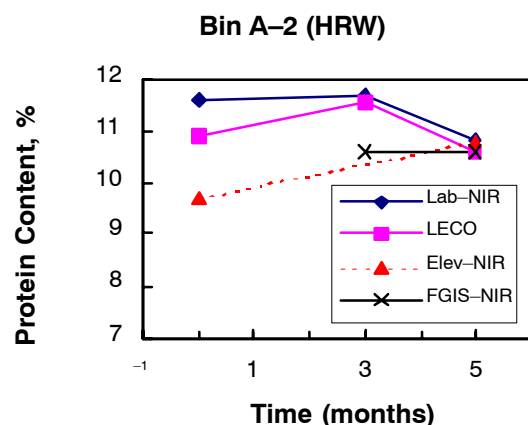
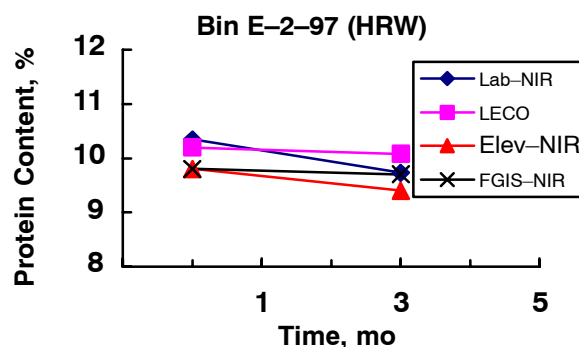
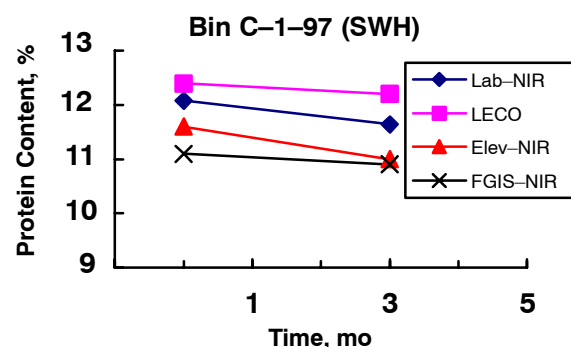


Figure 3. Protein contents measured over five months at one in-bin position, 1996.



(a)



(b)

Figure 4. Protein measurements from one position for wheat stored three months, 1997.

Figures 3 and 4b show cases with a large variation between the four instruments. The difference between instruments is also seen in tables 5 and 6, and was comparable to the difference between sample positions in the same bin (samples C1, C2, and C3 are progressively 1 m deeper positions in each bin). When the data for variation with position in the bin for each instrument (table 4) was compared to the variation in readings between the instruments (table 5) by ANOVA, there

Table 4. Protein variation with in-bin position, 1996.

Bin	Lab-NIR[a]		LECO		Elevator-NIRT		FGIS-NIRT	
	Average Std. Dev.	Average % C.V.	Average Std. Dev.	Average % C.V.	Average Std. Dev.	Average % C.V.	Average Std. Dev.	Average % C.V.
A	0.67	6.08	0.70	6.49	0.87	8.63	0.69	6.72
B	0.10	0.93	0.10	0.89	0.10	0.97	0.15	1.46
C	0.39	3.79	0.38	3.71	0.10	0.98	0.59	6.15
D	0.39	2.99	0.42	3.19	0.32	2.55	0.39	2.99
E	0.42	3.24	0.36	2.77	0.46	3.71	0.39	3.17
F	0.31	3.54	0.20	2.37	0.37	4.52	0.21	2.49
G	0.89	7.44	0.72	6.28	0.11	1.07	0.80	6.67
H	0.25	1.64	0.25	1.69	0.17	1.19	0.13	0.92
I	0.50	5.17	0.46	5.03	0.49	5.24	0.37	4.02
J	0.08	0.88	0.05	0.59	0.00	0.00	0.12	1.33
K	0.19	1.54	0.18	1.46	0.17	1.52	0.13	1.16
Average[b]	0.38	3.38	0.35	3.14	0.29	2.76	0.36	3.37

[a] Average, over time, of standard deviation, S.D. (% Protein), and coefficient of variation, % C.V., for 3-in. bin positions. Data was obtained for each instrument at two to four times per bin position, resulting in 6 to 12 samples for each instrument for each bin.

[b] Average % C.V. values in the bottom row were not significantly different ($\alpha = 0.05$).

Table 5. Protein variation between instruments, 1996 (averaged over time).

Bin	N ^[a]	Average Std. Dev.	Average % CV
A-1	3	0.435	3.9
A-2	3	0.555	5.1
A-3	3	0.528	5.4
B-1	2	0.596	5.5
B-2	2	0.622	5.8
B-3	2	0.675	6.2
C-1	2	0.156	1.4
C-2	2	0.206	2.0
C-3	2	0.173	1.7
D-1	4	0.316	2.4
D-2	4	0.257	2.0
D-3	4	0.302	2.3
E-1	2	0.294	2.3
E-2	2	0.329	2.7
E-3	2	0.327	2.5
F-1	3	0.375	4.3
F-2	3	0.446	5.1
F-3	3	0.247	2.9
G-1	2	0.408	3.8
G-2	2	0.464	4.0
G-3	2	0.547	4.6
H-1	2	0.410	2.8
H-2	2	0.218	1.5
H-3	2	0.424	2.8
I-1	2	0.384	3.8
I-2	2	0.120	1.3
I-3	2	0.354	3.8
J-1	2	0.347	3.9
J-2	2	0.100	1.1
J-3	2	0.377	4.2
K-1	3	0.404	3.4
K-2	3	0.372	3.1
K-3	3	0.401	3.4
Average		0.369	3.4

^[a] N is the number of times standard deviation values were calculated from averaging protein values from the different instruments.

was no significant difference between the means ($\alpha = 0.05$). The in-bin position in figure 3 had one of the larger differences between instruments of the samples at the early times. Figure 4b demonstrated large differences at both measurement times, while figure 4a is more typical of the 1997 results with a smaller variation between instruments.

A similar variation for positions in the same bin is demonstrated in table 4 where the standard deviations for average protein content were between 0.29 and 0.38% protein for all instruments. These average standard deviations were not significantly different for these instruments, suggesting that much of the variation was due to sampling variation, to which all of the instruments were uniformly subjected. In table 4, the individual standard deviations for each bin (i.e., each row in the table) were also usually very similar for all instruments, just as were the overall average standard deviations at the bottom of the table. Note that table 4 shows only how each instrument varied at one time for samples from three locations in the same bin; it shows average values from multiple times but does not include variability over time for the instrument.

Tables 5 and 6 show the variation between instruments for all of the in-bin positions. As with the three typical positions

Table 6. Protein variation between instruments, 1997 (averaged over time).

Bin	N ^[a]	Average Std. Dev.	Average % C.V.
A-1	2	0.283	2.40
A-2	2	0.254	2.61
A-3	2	0.302	3.29
B-1	2	0.254	2.21
B-2	2	0.206	1.75
B-3	2	0.211	1.77
C-1	2	0.588	5.06
C-2	2	0.376	3.32
C-3	2	0.419	3.87
D-1	2	0.219	1.92
D-2	2	0.281	2.50
D-3	2	0.350	3.17
F-1	2	0.342	3.69
F-2	2	0.279	2.82
F-3	2	0.318	3.04
Average		0.312	2.9

^[a] N is the number of times standard deviation values were calculated from averaging protein values from the different instruments.

in figure 2, there is considerable variation both between instruments and over time. The differences between instruments were consistent when averaged for the three time periods at each position with an overall average standard deviation of 0.369% protein content in 1996. This difference in 1997, 0.312% protein content, was slightly lower but was not significantly different ($\alpha = 0.05$). Note that the instruments generally measured a separate subsample taken from each of the samples, except that the two lab instruments worked from the same 40-g subsample as described in the Procedures.

LABORATORY MEASUREMENTS

The specimens stored in jars in the laboratory were not subject to the same sampling variation as the in-bin samples because of the limited volume. While these samples were still subject to any existing kernel to kernel variation in protein content, the smallest sample size was with the lab instruments, which worked from a 40-g sample, which would be at least 1000 kernels. A summary of statistics for the two instruments used with the laboratory specimens is shown in table 7. When compared to the same statistics for the in-bin samples from 1996 in table 2, the laboratory specimen standard deviations were a little higher for the combustion nitrogen analyzer, but very similar for the Lab-NIR instrument. There is no apparent reason that the individual instrument variation for the combustion nitrogen analyzer was greater with the samples from the laboratory specimens than it was with the bin samples. The standard deviations were also lower for the Lab-NIR compared to the combustion nitrogen instrument in the short 1997 season.

Table 7. Laboratory protein content statistics for both instruments averaged over time (8 measurements over 22 months).

Instruments	Std. Dev.	95%	% C.V. ^[a]
Lab-NIR	0.560	± 1.10	5.13
LECO	0.609	± 1.19	5.63
Both Instruments	0.581		5.35

^[a] Mean values of % C.V. for the two instruments were not significantly different ($\alpha = 0.05$).

Four typical plots of protein content versus time in figure 5 show occasional large differences between the two instruments and variation over time. These four samples generally follow a pattern of increasing early in the first year, then decreasing for the rest of that year, and following a similar up then down trend the second year. This general pattern is followed by many of the laboratory specimens, with minor variations between the particular specimens as seen in the four examples in figure 5 and suggests serious instrument and measurement errors for these two instruments.

The average of all of these deviations for the 22 laboratory samples is shown in figure 6. The plot for each instrument was obtained by subtracting the protein measurement for each sample from that sample's mean protein content from that instrument for all eight measurements over the 22-month period. Since the laboratory's standard procedure was to adjust the Lab-NIR instrument bias regularly based on the LECO, the similar trends are not surprising. This pattern also shows how the anecdotal reports that motivated this study (Suchan, 1997) could originate. There are several places on figure 5 that show a drop of greater than 1% protein and two places that drop by 2% or more. Someone submitting samples near the peak of these readings, then again near the low point would see a surprising drop of 1 to 2% in protein content due to this variability in the readings.

It must be noted that the elevator-NIR instrument showed changes that were the same magnitude as these laboratory instruments in figure 2, dropping 1.5% (C-1) and 1.4% (C-3) in the largest two cases. Tables 2 and 3 show that the elevator-NIR and lab-NIR were not significantly different so

that this magnitude of changes is not just a characteristic of these particular laboratory instruments used in the 22-month lab study. Only the FGIS-NIR showed significantly less variability among the near-IR instruments, and the combustion nitrogen instrument had significantly less variability than the two high variability near-IR instruments (Elevator-NIR and Lab-NIR) in only one of the two years.

CONCLUSIONS

In summary, these results showed the potential for large variations in protein content measurements for repeated samples taken from stored wheat. Data from the more typical year, 1996, showed the largest amount of variation between instruments and between different times. Different instruments registered differences of up to two percent protein content when measuring samples taken from the same position. The variations shown by individual instruments over time were even larger than that between instruments for the near-IR instruments, with the exception of the FGIS instrument, which had much less variation over time than the other near-IR instruments. Variation between different in-bin positions was not significantly different than the variation between instruments.

The average variation over time for the FGIS-NIR instrument was less than one-third of that for the other three instruments in 1996, and was also less than all other instruments in the short 1997 storage season. The greater consistency for the FGIS instrument was likely due to the rigorous maintenance and standardization procedures

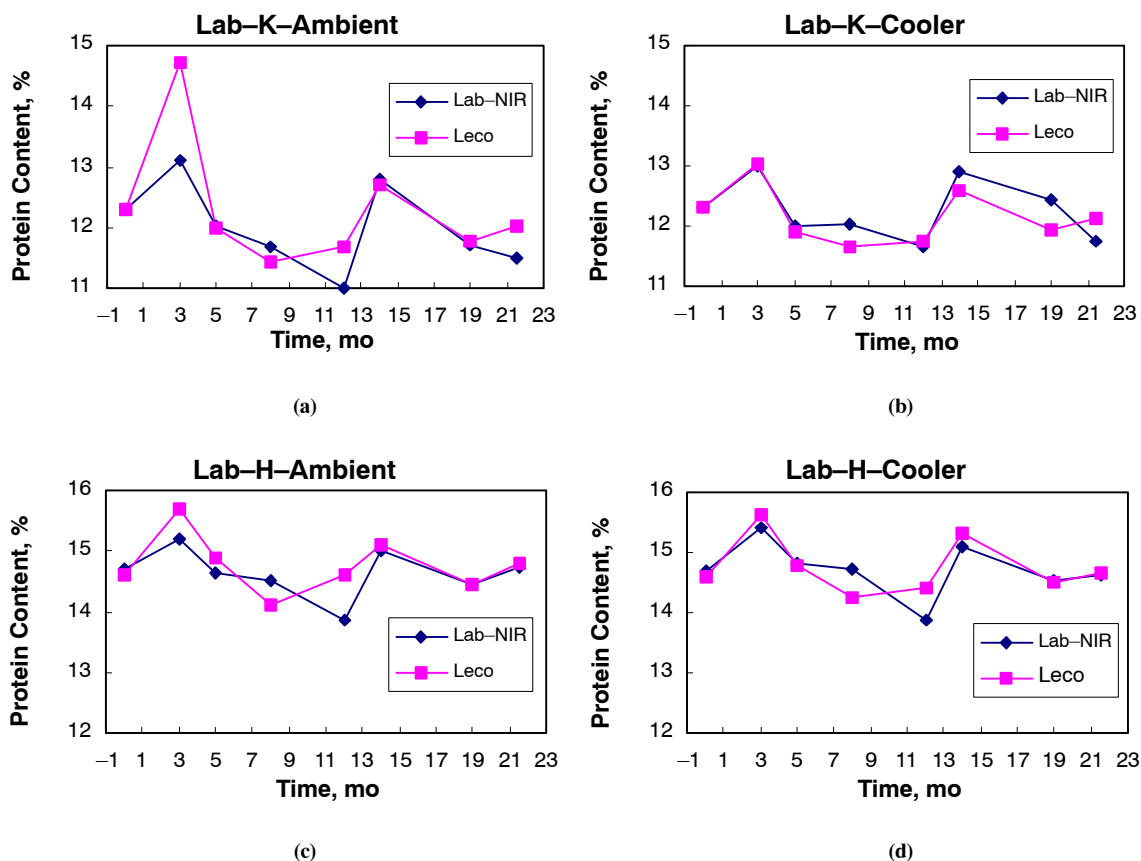


Figure 5. Protein measurements from laboratory specimens: wheat stored 22 months in glass jars.

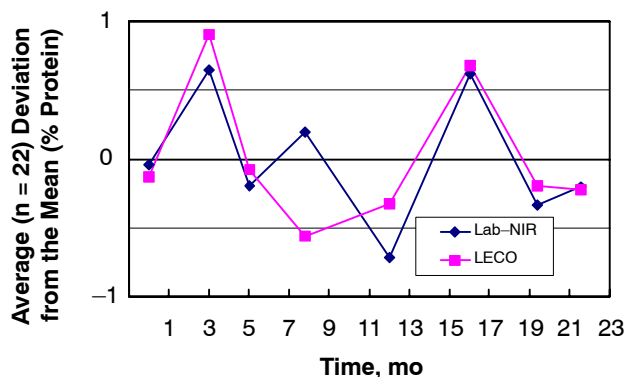


Figure 6. Average deviation from the 22-month mean protein content for 22 laboratory samples.

employed by FGIS (Pierce et al., 1996). These data suggest that a similar rigorous system of standardization and maintenance would be required to obtain the same consistency for other near-IR instruments used in the wheat marketing system. It shows the importance of laboratories being vigilant about maintaining the precision of their protein determination procedures. The following specific conclusions were based on the 1996 season, which was considered the more typical year, for protein measurements in on-farm wheat storage using four instruments (based on a 95% confidence interval):

1. The average variation between instrument measurements was $\pm 0.7\%$ protein content.
2. The average variation with position in the bin was $\pm 0.7\%$ protein content.
3. The FGIS instrument measurements had much less variation over time ($\pm 0.3\%$) than did the other three instruments, which varied by at least $\pm 0.8\%$ protein.

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